

## EFFECT OF ACTINOSPECTACIN (TROBICIN) IN EXPERIMENTAL SYPHILIS IN THE RABBIT

### II. IN SUBCLINICAL INCUBATING SYPHILIS\*

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The broad-spectrum antibiotic actinospectacin† may have general application in human therapy. Its effectiveness in the treatment of gonorrhoea has been reported by Willcox (1962) and by Laird and Taylor (1962). Earlier studies in this laboratory demonstrated its effect in experimental early lesion syphilis in the rabbit (Clark and Yobs, 1963). Patients with symptomatic gonorrhoea may also have inapparent incubating syphilis. This is a report of the effects of actinospectacin at the dosage level used in gonorrhoea and other human infections in subclinical incubating syphilis in the rabbit.

#### Experimental Methods

The methods for the determination of therapeutic effects in experimental lesion syphilis were used where applicable (Clark and Yobs, 1963). A fresh suspension of Nichols virulent strain *Treponema pallidum* was enumerated and diluted so that approximately 300 treponemes were contained in 0.2 ml. of one solution and 30,000 in 0.2 ml. of another. The clipped backs of fifteen young adult male rabbits were divided into six areas. One area on each of six animals was inoculated intracutaneously with 300 organisms, each rabbit being injected in a different area. Six other rabbits were inoculated with 30,000 treponemes per animal in the same manner. Three additional rabbits to serve as controls were inoculated in two areas each with 300 treponemes and in two different areas with 30,000 organisms per site. The inoculations were completed 45 minutes after the death of the donor.

Each of the twelve single inoculation rabbits were given one intramuscular injection of 20 mg./kg. Trobicin in water 4 days after the inoculation. The three multiple-site controls were not treated.

#### Results

All subjects survived the essential observations. The six 30,000 treponeme inoculation sites were

darkfield-positive on the ninth day of infection in the controls. This inoculum produced in the treated group a darkfield-positive papule in one rabbit after 14 days, in three after 22 days, in one after 28 days, and in another 45 days after infection (mean 25.5 days incubation to darkfield-positivity).

Incubation to darkfield-positivity for the sites inoculated with 300 treponemes ranged from 13 to 24 days, with 17 days the mean for the untreated rabbits. One of the treated group did not develop a lesion; the remaining five first developed darkfield-positive macules, one on day 22, one on day 24, and three on day 28 of incubation (mean 26 days).

In the treated groups, 300 treponemes produced no lesion in one rabbit. In one animal (No. 475) this inoculum produced a small primary papule 4 mm. in diameter  $\times$  0.5 mm. in height, which was proved darkfield-positive with difficulty on incubation day 28 and completely and permanently disappeared after 2 days. One of the six lesions from the 30,000 *T. pallida* inocula first emerged on the 45th day of incubation and eventually developed into a chancre.

All rabbits were VDRL slide test non-reactive 0, 1, and 2 weeks after inoculation, and the treated rabbits 3 weeks after inoculation. Two controls were reactive the third week, and the other the sixth week of incubation. 6 weeks after infection, seven of the twelve treated animals had become reactive, and at 12 weeks ten of the twelve were reactive.

Two treated animals inoculated with 300 *T. pallida* remained non-reactive. One of these showed no evidence of infection; the VDRL and TPI were non-reactive at 6 months, and nodal tissue was not infectious. The other (No. 475) developed the primary lesion previously described and a minute, confirmed secondary macule. VDRL and TPI results were negative at 12 weeks' incubation. The unexpected death of this animal precluded 6-month serological and tissue transfer data.

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† Supplied by Upjohn Co., Kalamazoo, Michigan, under trade name "Trobicin". Trade names are for identification only and do not represent an endorsement by the U.S. Public Health Service.

### Discussion

In man a single injection of 1.4 or 1.6 g. aqueous actinospectacin has been found efficacious in gonorrhoea in males (Willcox, 1962; Laird and Taylor, 1962) and in other infections (Lawson, 1961; Lindemeyer, Turck, and Petersdorf, 1962). While equivalent dosage levels did not cure experimental early lesion syphilis in the rabbit, 20 mg./kg. altered the clinical manifestations of the infection, and larger amounts cured the disease at that stage (Clark and Yobs, 1963). We have attempted to simulate experimentally the effect of this treatment for acute gonorrhoea on incubating syphilis acquired during the same exposure.

The actual numbers of *Treponema pallida* involved in the natural acquisition of syphilis is not known. As few as one treponeme in the rabbit (Magnuson, Eagle, and Fleischman, 1948) and ten in man (Magnuson, Thomas, Olansky, Kaplan, De Mello, and Cutler, 1956) have proved to be infectious. The subclinical incubation time is related to the number of treponemes introduced (Magnuson, and others, 1948). The incubation periods of 9 to 24 days to an observable darkfield-positive papule and of 21 to 42 days to sero-reactivity exhibited in the controls by the two inocula used here are of the order often reported clinically.

In the male acute gonorrhoeal symptoms are frequently presented for treatment by the third or fourth day following exposure. Therapy in this study was administered 4 days after infection.

Although the twelve inoculation control sites were all darkfield-positive, to ensure infectivity many more treponemes than the inoculations contained in this experiment must be introduced. Whether the rabbit which did not develop syphilis, after being injected with 300 *T. pallida* and treated on the fourth day of incubation, represented a failure to infect or a result of treatment could not be determined.

Only twelve rabbits were treated. The incubation time to darkfield-positivity in the six control sites and the six treated animals infected with 300 *T. pallida* overlapped in two instances, the ranges being 13 to 24 days (mean 17 days), and 22 days to infinity (mean for the five positives 26 days). The corresponding times for the 30,000 treponeme inocula were a range of 0 days (mean 9 days) for the controls, compared with a range of 14 to 45 days (mean 25.5) for the treated group. Three of the twelve treated rabbits, after extended incubation periods, produced small papules which were proved darkfield-positive only after repeated attempts. Two of these lesions immediately regressed. Except for the trauma of the collection of the many negative preparations from these sites, these two animals might not have exhibited trepo-

nemes. Similar insignificant primary manifestations in man could easily be neglected or overlooked. In view of the wide variations in this disease, these numbers are too small to be highly significant. However, the data indicate a definite prolongation of the subclinical incubation period and the increased possibility of inapparent early syphilis where actinospectacin is used in this manner.

If comparable effects occur in man, the diagnosis and epidemiology of early syphilis will become more difficult where the drug is used. Aqueous actinospectacin given intramuscularly is excreted rapidly, having a half-life of about 2 hours and a detectable level of 6 hours or so in the blood (Lawson, 1961), but it is presumed that longer acting forms will be produced. While extended blood levels could further confuse, they might solve the problem by aborting incubating syphilis.

### Summary

- (1) A study of the effect of actinospectacin, at the dosage level used in man in gonorrhoea and other disorders, on subclinical early experimental syphilis in the rabbit suggests that its effects may be: (a) A delay in the appearance of the primary lesion; (b) A masking of the signs and symptoms of infectious syphilis.
- (2) Implications of the effects, if applicable to human syphilis, are discussed.

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### La syphilis expérimentale chez le lapin traitée par l'actinospectacine (Trobicine)

#### II. Syphilis primaire asymptomatique

##### RÉSUMÉ

(1) On étudia l'effet de l'actinospectacine, dans un dosage comparable à ce qui est employé chez l'homme atteint de blennorragie ou de maladie semblable, sur la syphilis expérimentale asymptomatique chez le lapin. Ce médicament peut retarder l'apparition des lésions primaires ou déguiser les symptômes de la syphilis précoce.

(2) On discute les implications de ces effets selon leur application à la syphilis chez l'homme.